

Preventing High Fat Diet-induced Obesity and Improving Insulin Sensitivity through Neuregulin 4 Gene Transfer

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Supplementary Table S1

Supplementary Figure S1 and Figure Legend

Supplementary Figure S2 and Figure Legend

Supplementary Figure S3 and Figure Legend

Supplementary Figure S4 and Figure Legend

Supplemental Data

Table S1 Primer sets for real time PCR analysis of gene expression

Name	Forward sequence	Reverse sequence
<i>F4/80</i>	CCCCAGTGTCTTACAGAGTG	GTGCCAGAGTGGATGTCT
<i>Cd68</i>	CCATCCTTCACGATGACACCT	GGCAGGGTTATGAGTGACAGTT
<i>Cd11b</i>	ATGGACGCTGATGGCAATACC	TCCCCATTACCGTCTCCA
<i>Cd11c</i>	ACGTCAGTACAAGGAGATGTTGGA	ATCCTATTGCAGAATGCTTCTTAC
<i>Mcp1</i>	ACTGAAGCCAGCTCTCTTCCTC	TTCCCTCTGGGTAGCACAGAC
<i>Tnfa</i>	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
<i>Atgl</i>	CAACGCCACTCACATCTACGG	TCACCAGGTTGAAGGAGGGAT
<i>Adiponectin</i>	AGCCGCTTATATGTATCGCTCA	TGCCGTATAATGATTCTGTTGG
<i>Nrg4</i>	ATGCCAACAGATCACGAGC	TCTTCAGTGTCTCTGTGGCTG
<i>Ucp1</i>	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATT
<i>Ucp3</i>	ATGAGTTTGCCTCCATTCTG	GGCGTATCATGGCTTGAAAT
<i>Pgc1α</i>	GAAGTGGTAGCGACCAATC	AATGAGGGCAATCCGTCTCA
<i>Dio2</i>	AATTATGCCTCGGAGAACCG	GGCAGTTGCCTAGTGAAGGT
<i>Cidea</i>	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTCT
<i>Insulin1</i>	CACTCCTACCCCTGCTGG	ACCACAAAGATGCTGTTGACA
<i>Insulin2</i>	GCTTCTTCTACACACCCATGTC	AGCACTGATCTACAATGCCAC
<i>Pepck</i>	AAGCATTCAACGCCAGGTT	GGCGAGTCTGTCAGTTCAAT
<i>G6pase</i>	CGACTCGCTATCTCCAAGTGA	GTTGAACCAGTCTCCGACCA
<i>Srebp-1c</i>	CCCTGTGTACTGGCCTTT	TTGCATGTCTCCAGAAGTG
<i>Acc-1</i>	GCCTCTCCTGACAAACGAG	TGACTGCCAAACATCTCTG
<i>Fas</i>	AGAGATCCCAGACGCTTCT	GCCTGGTAGGCATTCTGTAGT
<i>Scd-1</i>	TTCTTACACGACCACCACCA	CCGAAGAGGCAGGTGTAGAG
<i>Cyp7a1</i>	AACGGGTTGATTCCATACCTGG	GTGGACATATTCCCCATCAGTT
<i>Hmgcr</i>	CTTGTGGAATGCCTGTGATTG	AGCCGAAGCAGCACATGAT
<i>Abca1</i>	AAAACCGCAGACATCCTCAG	CATACGAAACTCGTCACCC
<i>Pparγ1</i>	TTTTCCGAAGAACCATCCGATT	ATGGCATTGTGAGACATCCCC
<i>Pparγ2</i>	TCGCTGATGCACTGCCTATG	GAGAGGTCCACAGAGCTGATT
<i>Cd36</i>	CCTTAAAGGAATCCCCGTGT	TGCATTGCCAATGTCTAGC
<i>Fabp4</i>	AAGGTGAAGAGCATAACCC	TCACGCCCTTCATAACACATTCC
<i>Mgat1</i>	TGGTGCCAGTTGGTCCAG	TGCTCTGAGGTCGGGTTCA
<i>Ppara</i>	TGTGAAATATGTGGGGACAA	AATCTTGCAGCTCCGATCAC
<i>Cpt1a</i>	CTCCGCTTGAGCCATGAAG	CACCAAGTGTGATGCCATTCT
<i>Cpt1b</i>	GGTCTCTTCTCAAGGTCTG	CGAGGATTCTCTGGAACACTGC
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTG	TGTAGACCATGTAGTTGAGGTCA

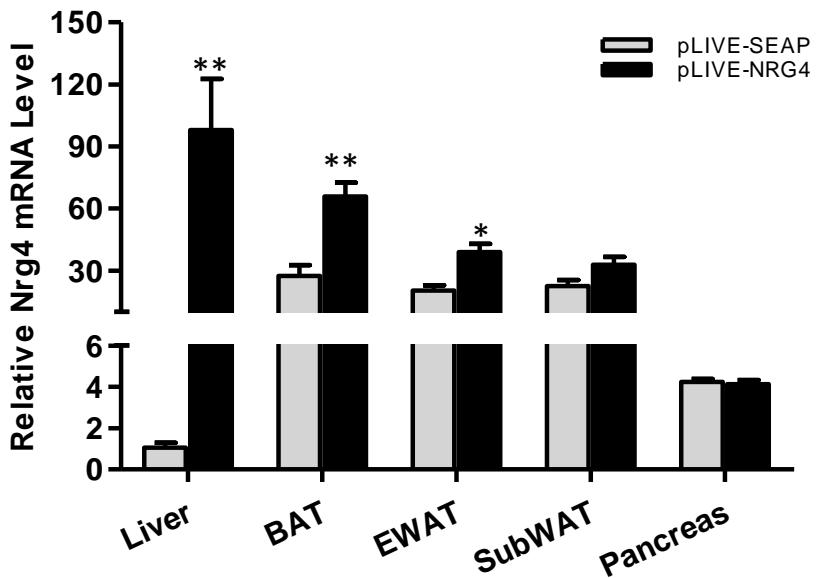


Figure S1 *Nrg4* gene expression in different tissues. Eight-week-old C57BL/6 male mice were hydrodynamically injected via tail vein of 20 µg of pLIVE-NRG4 or pLIVE-SEAP control pasmid DNA and fed a HFD for 9 weeks. At the end of experiment, total RNA was extracted from liver, EWAT, SubWAT, BAT, pancreatic tissues, and the relative mRNA levels of *Nrg4* gene were determined by real-time or regular PCR. *P<0.05, **P<0.01 compared to that of control animals injected with pLIVE-SEAP ($n=5$).

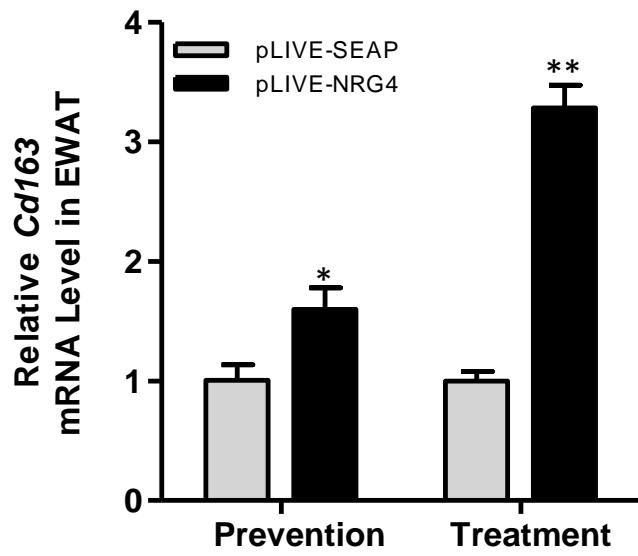


Figure S2 *Nrg4* gene transfer increased M2 macrophage marker *Cd163* in EWAT.

At the end of experiment, total RNA was extracted from EWAT and the relative mRNA levels of *Cd163* gene were determined by real-time. *P<0.05, **P<0.01 compared to that of control animals injected with pLIVE-SEAP ($n=5$).

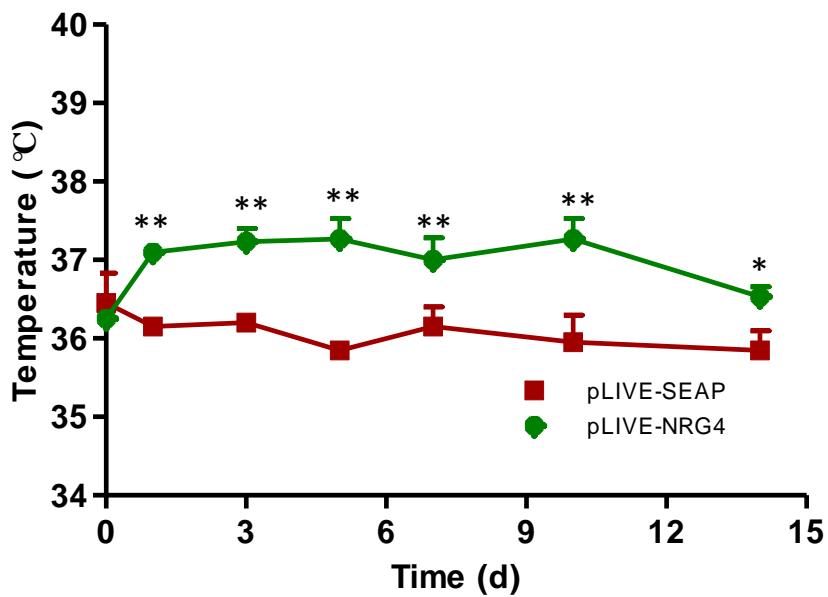


Figure S3 *Nrg4* gene transfer increased mice body temperature

After C57BL/6 male mice were hydrodynamically injected via tail vein of 20 μ g of pLIVE-NRG4 or pLIVE-SEAP control plasmid DNA. Rectal temperature was measured at desirable time after gene delivery. * $P<0.05$, ** $P<0.01$ compared to pLIVE-SEAP group. (n=5)

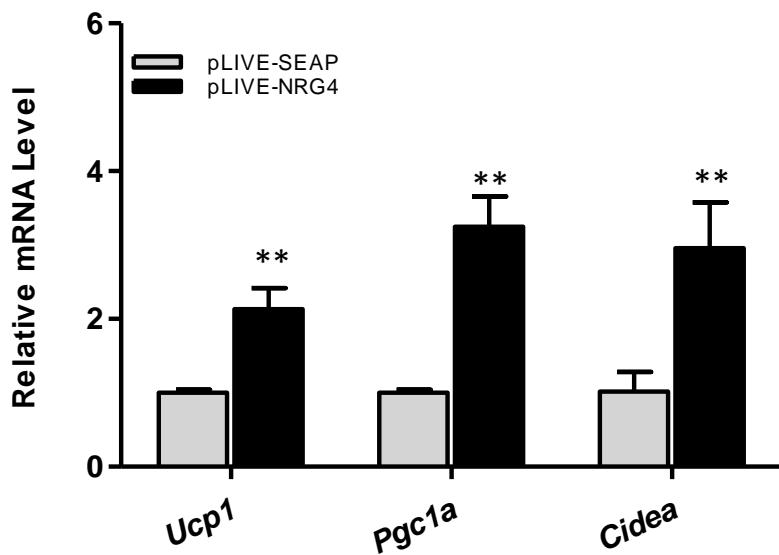


Figure S4 *Nrg4* gene transfer increased browning maker genes in inguinal WAT.

At the end of experiment, total RNA was extracted from inguinal WAT and the relative mRNA levels of thermogenic genes expression were determined by real-time. **P<0.01 compared to that of control animals injected with pLIVE-SEAP ($n=5$).